Mar 16, 2023

Viral PEG purification

DOI
https://dx.doi.org/10.17504/protocols.io.n92ldp6dxl5b/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.n92ldp6dxl5b/v1

Protocol Citation: sarah.schulz 2023. Viral PEG purification. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldp6dxl5b/v1

Protocol status: Working
We use this protocol and it's working

Created: March 15, 2023

Last Modified: March 21, 2023

Protocol Integer ID: 78835

Keywords: viral peg purification protocol, viral particle purification, peg percipitation, purification

Abstract

Protocol for viral particle purification via PEG percipitation

Materials

PBS, 0.45 µm syringe filter, 10% w/v PEG-8000, 0.5 M NaCl, 10 mM CaCl2, 50 mM MgCl2, DNase I, RNase A

1 Add 30 ml of ice cold PBS to 3 g of faecal sample and mix by vortexing at high speed for 5 min, then keep on ice

2 Centrifuge sample at 5000 rpm for 10 min at 4°C and transfer the supernatant to a clean 50 ml centrifuge tube

3 Filter the supernatant through a 0.45 µm syringe filter

4 Add 10 % 10% w/v PEG-8000 in saline (0.5 M NaCl) to filtrate, mix by inverting and incubate at 4°C overnight

5 Centrifuge sample at 5000 rpm for 20 min at 4°C and discard the supernatant.

6 Re-suspend pellet in 200 µl PBS and transfer it to a 1.5ml Eppendorf tube

7 Add an equal volume of chloroform to the sample and mix gently before centrifuging it at 2500 rpm for 5 min at room temperature

8 Transfer the aqueous phase into a new and sterile 1.5 ml tube

9 Add 40 µl of 10 mM CaCl2 and 50 mM MgCl2 to the sample, as well as 10 U of DNase and 10 U of RNase A

10 Incubate samples at 37°C for 2 h and then place them in a heatblock at 70°C for 10 min

11 Store at 4°C